SUPPLEMENTARY DATA

Dynamic Energy Dependency of *Chlamydia trachomatis* on Host Cell Metabolism during Different Stages of Intracellular Growth: Possible role of Sodium -based energetics in Chlamydial ATP generation

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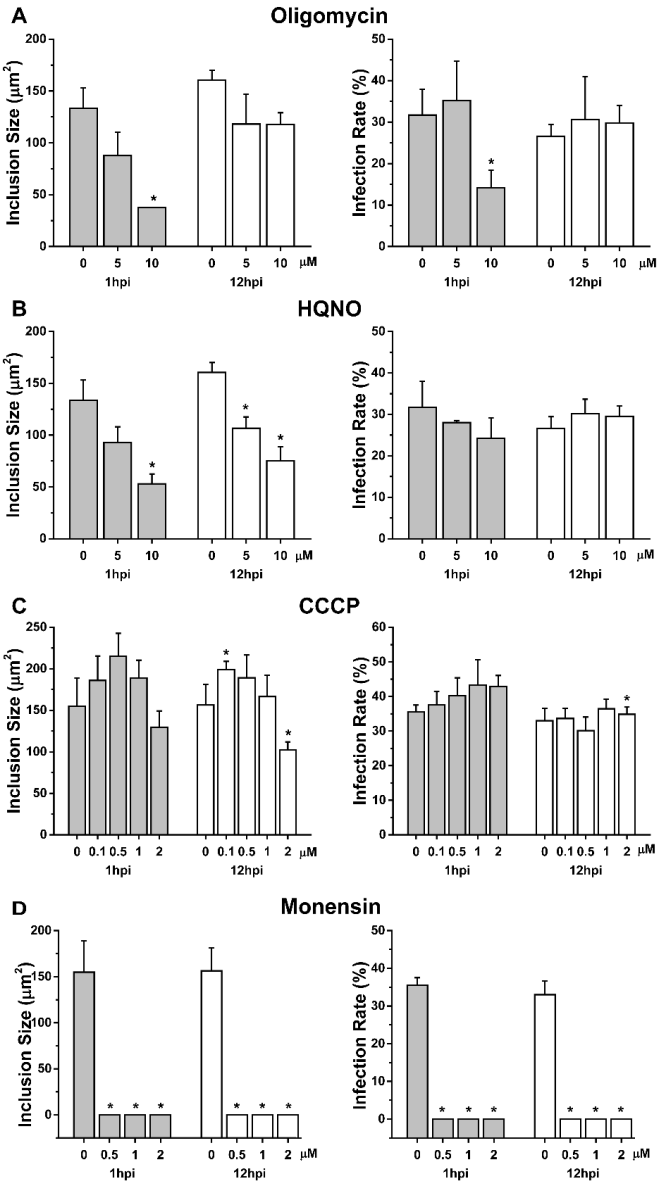

Figure S1. Effects of respiratory chain inhibitors and ionophores on Chlamydial infection in HeLa cells. The inclusion size and the infection rate of *C. trachomatis*-infected HeLa cells were evaluated at 36 hpi by analyzing the immunofluorescence of cells cultures stained with anti-MOMP antibodies and secondary fluorescently labeled antibodies. 200 inclusions were measured per condition per experiment using Image J. A) Oligomycin 0, 5, 10 μM; B) HQNO 0, 5, 10 μM; C) CCCP 0, 0.1, 0.5, 1, 2 μM; and D) Monensin 0, 0.5, 1, 2 μM were added at 1 hpi or 12 hpi. Error bars indicate the standard deviation of the mean, n = 3. Asterisk denotes significance from vehicle-treated control. *, $p<0.05$.

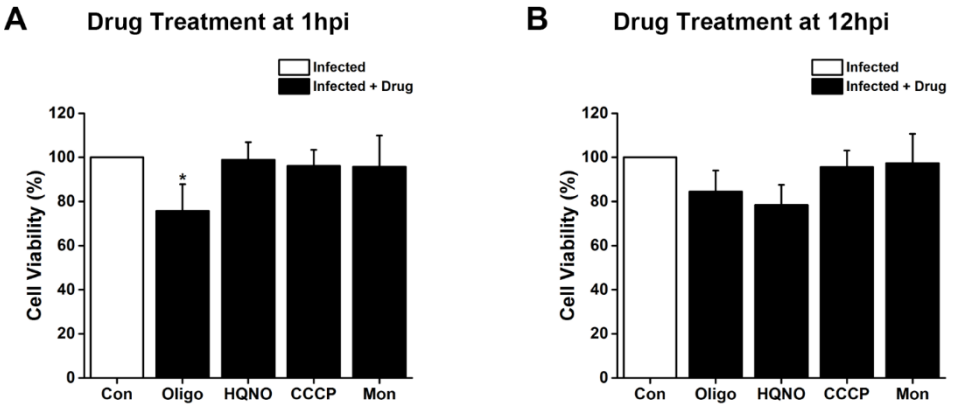

Figure S2. Viability of *C. trachomatis*-infected HeLa cells. Infected HeLa cells were treated with 10 μM Oligomycin A (Oligo), 10 μM HQNO, 2 μM CCCP or 2 μM monensin (Mon) at 1 hpi or 12 hpi and stained with HEMA 3 staining at 36 hpi. The number of cells per field were quantified in at least 5 random fields per sample. Results are expressed as mean ± S.D. of 3 different experiments per condition. Asterisk denotes significance from vehicle-treated control (Con). *$p<0.05$.

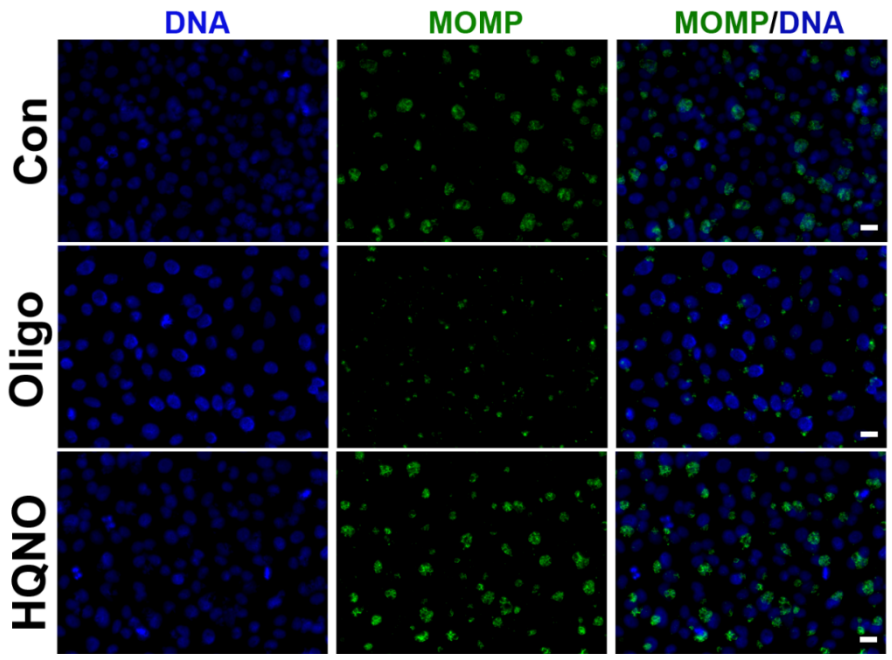

Figure S3. Immunofluorescence images of *Chlamydia trachomatis*-infected HeLa cells treated with respiratory inhibitors at 1 hpi. Representative images of *C. trachomatis*-infected HeLa cultures treated with 10 μM Oligomycin A (Oligo) or 10 μM HQNO at 1 hpi and immunostained with anti-MOMP antibodies (green) at 36 hpi. DNA is visualized with Hoechst 33342 (blue). Scale bars represent 20 μm. MOMP/DNA merged images correspond to those in Fig. 4A. Fluorescent channels are shown separately for clarity.

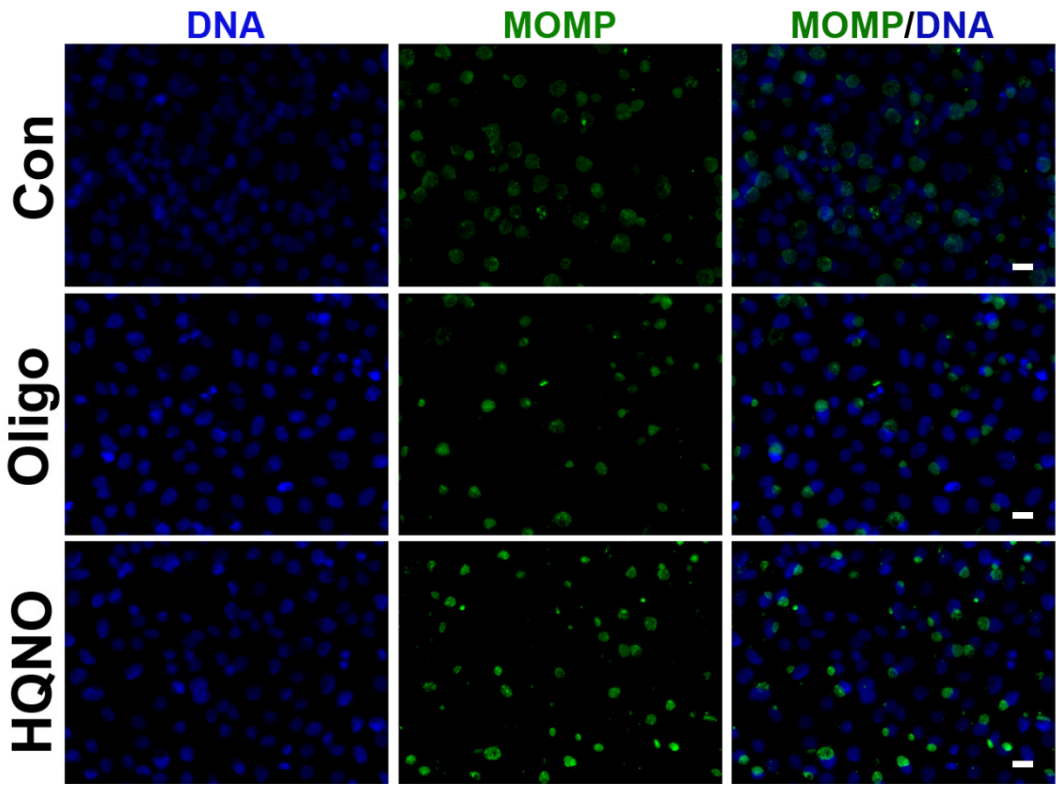

Figure S4. Immunofluorescence images of *C. trachomatis*-infected HeLa cells treated with respiratory inhibitors at 12 hpi. *C. trachomatis*-infected HeLa cells were treated with 10 μM of oligomycin A (Oligo) or HQNO at 12 hpi and immunostained with anti-MOMP antibodies (green) at 36 hpi. DNA was labeled with Hoechst 33342 (blue). Scale bars indicate 20 μm. Merged images correspond to those in Fig. 4B.

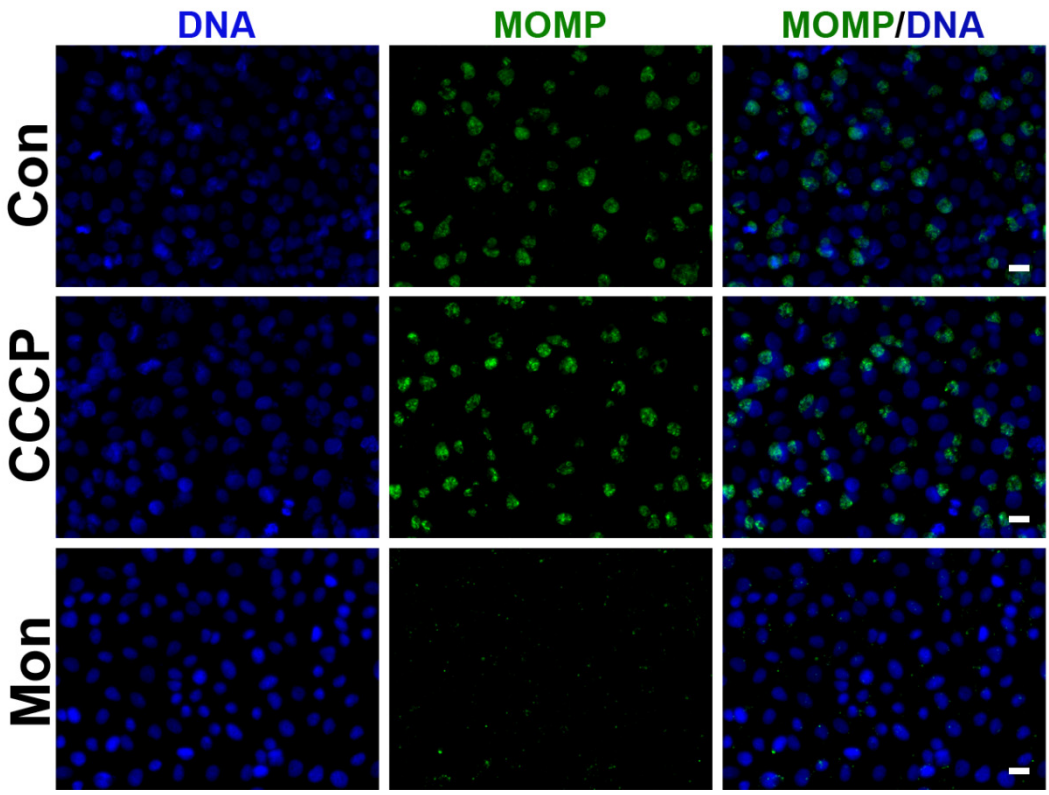

Figure S5. *C. trachomatis*-infected HeLa cells treated with ionophores at 1 hpi. Representative immunofluorescent images of *Chlamydia trachomatis*-infected HeLa cultures treated with 2 μM monensin (Mon) or CCCP at 1 hpi and immunostained with anti-MOMP antibodies (green) at 36 hpi. DNA is visualized with Hoechst 33342 (blue). MOMP/DNA merged images are the same shown in Fig. 6A. Scale bars = 20 μm.

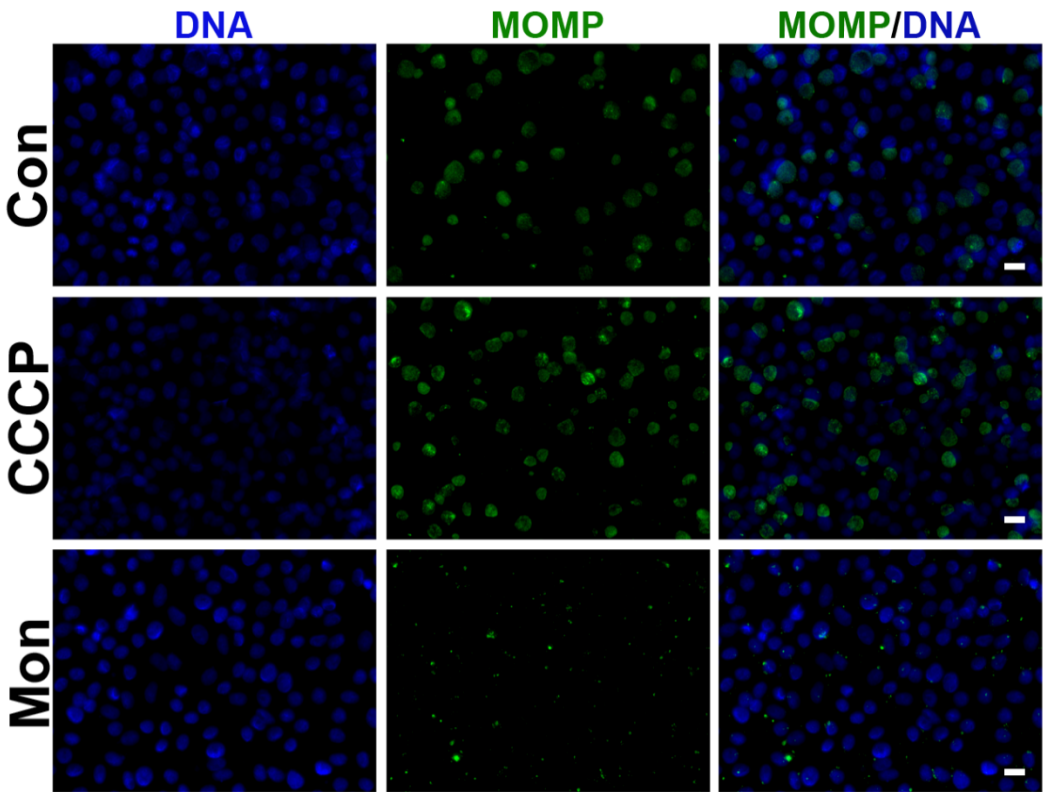

Figure S6. *C. trachomatis*-infected HeLa cells treated with ionophores at 12 hpi. Representative immunofluorescent images of *C. trachomatis*-infected HeLa cultures treated with 2 μM monensin (Mon) or CCCP at 12 hpi and immunostained with anti-MOMP antibodies (green) at 36 hpi. DNA was labeled with Hoechst 33342 (blue). Merged images are the same as in Fig. 6B. Scale bars = 20 μm.